Mustard Oil (Allyl Isothiocyanate) Toxicity & Teratogenicity studies in Avian Embryos FDA Contract #72-345 No Date

w 20

MUSTARD OIL
(allyl isothiocyanate)

TOXICITY and TERATOGENICITY STUDIES
in Avian Embryos

FDA CONTRACT #72-345

B.L. Reid

Department of Poultry Science
University of Arizona, Tucson, Arizona 85721

STUDIES on the TOXICITY and TERATOGENICITY of MUSTARD OIL (allyl isothiocyanate)

SUMMARY and CONCLUSIONS

Mustard oil (allyl isothiocyanate) was toxic to avian embryos at levels of 1.0 and 0.6 mg/kg administered in the air cell at 0 or 96 hrs, respectively. Yolk administration of this material in 96 hr embryonated eggs resulted in significant increases in embryo mortality with doses as low as 0.04 mg/kg.

The occurrence of abnormal embryos and those showing head, skeletal, visceral and limb abnormalities were not increased with the administration of mustard oil in any of the four test protocols. This substance was not teratogenic under the conditions employed in these studies.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #72-345 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (pre-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Mustard oil (allyl isothiocyanate) was solublized in absolute alcohol for use in the test protocols. The maximum dose level of 16 mg/kg was obtained with a solution of 16 mg/ml.

MORTALITY

The mortality data obtained in the four test protocols are shown in Tables 1 - 4. Chi-square analyses of these data indicate that mustard oil was toxic to chicken embryos at relatively low concentrations (Table 5). Air cell administration of mustard oil at either 0 or 96 hrs resulted in significant increases in embryo mortality at dose levels of 1.0 and 0.6 mg/kg, respectively. Levels as low as 0.04 mg/kg resulted in in a significant increase in embryo mortality when injected into the yolk at 96 hrs incubation.

Linear regression analyses of the mortality data employing log dose as the independent variable and probit of mortality as the dependent variable indicated a significant relationship ($P < 0.05$) for only the air cell-96 hr series with an LD-50 estimate of 0.4 mg/kg (Table 6). The data obtained for yolk administration at 0 hrs indicated a linear relationship between log dose and probit of mortality which was significant at the 0.1 level of probability (Table 6).

TERATOLOGY

The occurrence of abnormal embryos and those showing H-S-V-L abnormalities in the four test protocols are shown in Tables 1 - 4. Chi-square analyses of these data failed to indicate that either the incidence of abnormal embryos or those showing H-S-V-L abnormalities

we significantly different from the solvent controls (Tables 7 & 9). Probit analyses of these data failed to demonstrate a statistically significant linear relationship between log dose and probit of abnormality incidence. The individual teratogenic findings are shown in Table 10. Under the conditions employed in these studies, mustard oil (allyl isothiocyanate) was not found to be teratogenic in chicken embryos.

TABLE

MUSTARD OIL (ALLYL ISOTHIOCYANATE)
in ALCOHOL, DEHYDRATED
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
1.0 1.0	135	28.88	39	0.74	1	1.48	2	0.74	1			0.74	1								
0.6 0.6	100	12.00	12	2.00	2	2.00	2	2.00	2												
0.2 0.2	99	14.14	14	1.01	1	1.01	1	1.01	1												
0.08 0.08	100	9.00	9	1.00	1	0.00	0													1.00	1
0.04 0.04	100	12.00	12	0.00	0	0.00	0														
0.0 0.0	135	14.07	19	2.96	4	2.22	3	0.74	1			1.48	2							0.74	1
drilled drilled	99	17.17	17	1.01	1	1.01	1	1.01	1												
untreated untreated	301	11.62	35	0.00	0	0.00	0														

SUMMARY - ALL DOSE LEVELS

534	16.10	86	0.94	5	0.94	5	0.75	4			0.19	1							0.19	1

TABLE 2

MUSTARD OIL (ALLY[illegible]OTHIOCYANATE)
in ALCOHOL, DEHYDRATED
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
1.0	120	100.00	120	0.00	0	0.00	0														
0.6	120	89.16	107	0.83	1	0.83	1							0.83	1					0.83	1
0.2	157	28.02	44	1.27	2	1.27	2					1.27	2								
0.08	119	28.57	34	2.52	3	2.52	3	1.68	2			0.84	1					0.84	1		
0.04	120	10.83	13	0.00	0	0.00	0														
0.0	157	23.56	37	1.91	3	1.27	2	1.27	2							0.63	1				
drilled																					
lled	109	10.09	11	2.75	3	1.83	2	1.83	2							0.91	1			0.91	1
Untreated																					
eated	301	11.62	35	0.00	0	0.00	0														

SUMMARY - ALL DOSE LEVELS

636	50.00	318	0.94	6	0.94	6	0.31	2		0.47	3	0.16	1		0.16	1	0.16	1

TABLE 3

MUSTARD OIL (ALL SOTHIOCYANATE)
IN ALCOHOL, DEHYDRATED
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Struc-tural %	Struc-tural #	Toxic Response %	Toxic Response #	Functiona %	Functiona #
0.60	99	59.59	59	0.00	0	0.00	0														
0.20	97	42.26	41	0.00	0	0.00	0														
0.12	99	47.47	47	3.03	3	2.02	2	1.01	1			1.01	1			1.01	1			1.01	1
0.08	99	42.42	42	2.02	2	0.00	0									2.02	2				
0.04	99	39.39	39	0.00	0	0.00	0														
0.032	37	62.16	23	0.00	0	0.00	0														
0.00	78	80.76	63	0.00	0	0.00	0														
Pierced ierced																					
Untreated treated	301	11.62	35	0.00	0	0.00	0														

SUMMARY - ALL DOSE LEVELS

530	47.36	251	0.94	5	0.38	2	0.19	1			0.19	1		0.57	3			0.19	1

TABLE 4

MUSTARD OIL (ALLYLISOTHIOCYANATE)
in ALCOHOL, DEHYDRATED
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functiona %
16.00	40	97.50	39	0.00	0	0.00	0													
6.00	40	97.50	39	0.00	0	0.00	0													
3.20	40	55.00	22	0.00	0	0.00	0													
1.60	40	55.00	22	0.00	0	0.00	0													
0.80	40	22.50	9	0.00	0	0.00	0													
0.60	60	96.66	58	0.00	0	0.00	0													
0.20	60	98.33	59	0.00	0	0.00	0													
0.12	59	98.30	58	0.00	0	0.00	0													
0.08	97	77.31	75	0.00	0	0.00	0													
0.04	60	56.66	34	0.00	0	0.00	0													
0.00	137	37.95	52	1.45	2	1.45	2	1.45	2											
ierced	100	17.00	17	0.00	0	0.00	0													
treated	301	11.62	35	0.00	0	0.00	0													

SUMMARY - ALL DOSE LEVELS

536	77.43 415	0.00	0	0.00	0							

TABLE 5

MUSTARD OIL (ALLYL ISOTHIOCYANATE)
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
0.032	-	-	3.674	-
0.040	0.073	6.619* (less)	28.915*(less)	5.202*
0.080	0.967	0.645	25.017*(less)	33.889*
0.120	-	-	19.167*(less)	58.566*
0.200	0.031	0.599	25.008*(less)	59.409*
0.600	0.073	114.647*	8.170*(less)	55.969*
0.800	-	-	-	2.626
1.000	7.927*	158.726*	-	-
1.600	-	-	-	3.029
3.200	-	-	-	3.029
6.000	-	-	-	41.591*
16.000	-	-	-	41.591*
All Doses(DF)	23.661*(5)	358.568*(5)	44.063*(6)	218.050*(10)

* Probability $<$ 0.05 - 0.005.

TABLE 6

MUSTARD OIL (ALLYL ISOTHIOCYANATE)
PROBIT ANALYSES - MORTALTIY

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs $P < 0.1$	96 hrs
LD-30	NS	0.3	0.04	NS
LD-50	NS	0.4	0.28	NS
LD-70	NS	0.5	2.18	NS
LD-90	NS	0.6	42.89	NS

TABLE 7

MUSTARD OIL (ALLYL ISOTHIOCYANATE)
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
0.032	-	-	0.000	-
0.040	1.503	0.878	0.000	0.028
0.080	0.329	0.005	0.298	0.225
0.120	-	-	0.930	0.025
0.200	0.317	0.000	0.000	0.028
0.600	0.002	0.056	0.000	0.028
0.800	-	-	-	0.007
1.000	0.815	0.878	-	-
1.600	-	-	-	0.007
3.200	-	-	-	0.007
6.000	-	-	-	0.007
16.000	-	-	-	0.007
All Doses(DF)	4.894(5)	5.759(5)	11.059(6)	7.848(10)

TABLE 8

MUSTARD OIL (ALLYL ISOTHIOCYANATE)
PROBIT ANALYSES - ABNORMALITIES

Air Cell		Yolk	
0 hrs	96 hrs	0 hrs	96 hrs
NS	NS	NS	NS

TABLE 9

MUSTARD OIL (ALLYL ISOTHIOCYANATE)
CHI-SQUARE ANALYSES of HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
0.032	-	-	0.000	-
0.040	0.833	0.275	0.000	0.028
0.080	0.833	0.052	0.000	0.225
0.120	-	-	0.298	0.025
0.200	0.039	0.253	0.000	0.028
0.600	0.116	0.055	0.000	0.028
0.800	-	-	-	0.007
1.000	0.254	0.275	-	-
1.600	-	-	-	0.007
3.200	-	-	-	0.007
6.000	-	-	-	0.007
16.000	-	-	-	0.007
All Doses(DF)	4.919(5)	3.556(5)	10.317(6)	7.848(10)

TERATOGEN FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Drilled - 0 hrs		99	1	1	exencephaly; abnormal shortening_maxilla
Drilled - 96 hrs		109	3	1	anophthalmia; abnormal shortening-maxilla; dysgnathia-beak
				1	hemorrhage-head; dwarfism
				1	cachexia
Air Cell - 0 hrs	1.0 mg/kg	135	1	1	agenesis-head; celosomia-abdomen
	0.6	100	2	1	anophthalmia; exencephaly; agenesis-maxilla
				1	exencephaly
	0.2	99	1	1	anophthalmia; exencephaly; abnormal shortening-maxi dysgnathia-beak
	0.08	100	1	1	cachexia
	0.0	135	4	1	anophthalmia
				1	celosomia-abdomen
			1	1	cachexia
				1	fusion failure-abdomen
Air Cell - 96 hrs	0.6	120	1	1	cachexia; abnormal curvature-toe
	0.2	157	2	2	celosomia-abdomen
	0.08	119	3	1	excencephaly
				1	anophthalmia; celosomia-abdomen
				1	hypopigmentation-down
	0.0	157	3	1	agenesis-down
				2	buphthalmia

MUSTARD OIL (allyl isothiocyanate) in ALCOHOL, DEHYDRATED

TERATOGEN FINDINGS

TREATMENT		TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Yolk - 0 hrs	0.12 mg/kg	99	3	1	cachexia
				1	anophthalmia; dysgnathia-beak; dwarfism
				1	fusion failure-abdomen
	0.08	99	2	1	agenesis-down
				1	dwarfism
Yolk - 96 hrs	0.0	137	2	1	dysgnathia-beak
				1	abnormal shortening-maxilla; dysgnathia-beak